Note on the Relation of Spermatozoa to Electrolytes and its bearing on the Problem of Fertilization.

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In June 1913, a number of large male Luidia were obtained at Plymouth, and to all external appearances the gonads appeared to be perfectly ripe; when, however, the spermatic fluid in sea-water was examined under the microscope, the spermatozoa were found to be quite motionless, and repeated experiments showed them to be quite incapable of fertilising ripe eggs of the same species. It was found, however, that the addition of a few drops of $\frac{N}{10}$ NaOH to a suspension of sperm in sea-water immediately caused active movement. When such "activated" sperm was added to eggs from the same female as before, every egg was quickly fertilised and large numbers of healthy larvæ were obtained from the culture.[1]

It might be supposed that the activation of the sperm by alkali was essentially an analogous phenomenon to the ripening of starfish eggs, which is dependent upon the alkalinity of the surrounding sea-water. It should be noted, however, that the spermatozoa began to move immediately the alkalinity of the solution was raised. Again, the following experiments dealing with the behaviour of Echinoid sperm, support the conclusion that active movement is dependent upon the alkalinity of the surrounding fluid, even when the spermatozoa are known to be perfectly ripe.

[1] An essentially similar phenomenon was observed this year in the case of several male Asterias glacialis.

(1) The Behaviour of Echinus acutus Spermatozoa in Acid Sea-water.

Solution.	Behaviour of sperm.
(1) Pure sea-water . .	Active movements for at least two hours.
(2) 5 c.c. sea-water + 1 drop N/10 HCl.	Active movement, which almost ceased after thirty minutes.
(3) 5 c.c. sea-water + 2 drops N/10 HCl.	Active movement, which almost ceased after seventeen minutes.
(4) 5 c.c. sea-water + 3 drops N/10 HCl.	Active movement, greatly reduced after four minutes, very slow after fourteen minutes.
(5) 5 c.c. sea-water + 4 drops N/10 HCl.	No movement (no agglutination).
(6) 5 c.c. sea-water + 5 drops N/10 HCl.	
(7) 5 c.c. sea-water + 6 drops N/10 HCl.	
(8) 5 c.c. sea-water + 7 drops N/10 HCl.	

(2) The Behaviour of E. acutus Spermatozoa in Alkaline Sea-water.

Solution.	Behaviour of sperm.
(1) Sea-water (which was faintly alkaline).	Prolonged active movement.
(2) 5 c.c. sea-water + 1 drop N/10 NaOH.	Active movement.
(3) 5 c.c. sea-water + 2 drops N/10 NaOH.	
(4) 5 c.c. sea-water + 3 drops N/10 NaOH.	
(5) 5 c.c. sea-water + 4 drops N/10 NaOH.	
(6) 5 c.c. sea-water + 5 drops N/10 NaOH.	Movement slower, some agglutination.
(7) 5 c.c. sea-water + 6 drops N/10 NaOH.	
(8) 5 c.c. sea-water + 7 drops N/10 NaOH.	Movement slower, considerable agglutination.

The effect of excess of alkali in the water is to bring the spermatozoa to rest by agglutination. About a minute after the addition of sperm to such a solution, the liquid becomes cloudy, and if examined under the microscope, it is found that most of the spermatozoa are inactive and aggregated into clumps—those which are agglutinated are usually active. This agglutination phenomenon is never produced by acids.

Similar experiments were performed last year, using artificial sea-water free from carbonates and phosphates. Identical results were obtained, but smaller quantities of acid and alkali were required than in the original experiments.

From these experiments it appears that spermatozoa are much more sensitive in their behaviour to acid than to alkali, and an attempt was made to determine whether a certain concentration of OH is necessary in order that the sperm may exhibit its usual activity.

It has been shown that the spermatozoa did not move in acid sea-water; if this cessation of movement is due to the absence of hydroxyl-ion in the surrounding medium, then by increasing the alkalinity of the water movement of the sperm should again take place. This is, undoubtedly, what takes place, as is shown by the following experiments:

(1) A drop of sperm was put into samples of sea-water containing various concentrations of N/10 HCl (such as previous experiments had shown caused cessation of movement); when all movement had ceased in the solutions one or more drops of N/10 NaOH were added, and the behaviour of the sperm watched under the microscope.

Solution.	No. of drops N/10 NaOH added.	Behaviour of sperm.
5 c.c. sea-water + 1 drop N/10 HCl.	1	Active movement re-commenced. No agglutination.
5 c.c. sea-water + 2 drops N/10 HCl.	1	Rapid movement began. Some agglutination.
5 c.c. sea-water + 3 drops N/10 HCl.	2	Movement greatly increased. Some agglutination.
5 c.c. sea-water + 4 drops N/10 HCl.	1–6	Sperm did not move on addition of the alkali, but rapid agglutination of all the spermatozoa took place.
5 c.c. sea-water + 5 drops N/10 HCl.		
5 c.c. sea-water + 6 drops N/10 HCl.		

This experiment was repeated several times with the same results, viz. that sperm which is motionless in "acid" sea-water, can be rendered motile by the addition of alkali, if the concentration of acid used in the experiment is not too great. Agglutination of spermatozoa is only caused by alkali, and not by acid, although the latter be of relatively high concentration. The cessation of movement in acid and that in alkaline sea-water are two separate phenomena; in the former case the spermatozoa never agglutinate, and the addition of alkali can remove the effect of the acid. In alkaline solutions, however, only relatively high concentrations produce cessation of movement, and this is closely connected with the agglutination phenomenon; the agglutination by alkali cannot be reversed by the addition of acid.

The behaviour of the spermatozoa of Echinus in acid and alkaline solutions is apparently identical with that of Nereis and Arbacia sperm as described by F. R. Lillie (1). This author, however, does not mention that the effect of acid can be removed by the addition of alkali. This fact, however, leads to a consideration of the possibility that the movement of spermotozoa is dependent upon the electromotive properties of the cell and of its medium. This suggestion is supported by two series of experiments: (1) The behaviour of spermatozoa in an electric field, (2) the behaviour of spermatozoa in the presence of trivalent ions.

(1) The Behaviour of Spermatozoa in an Electric Field.

If spermatozoa are suspended in an isotonic cane-sugar solution, their activity is lost, but if a trace of alkali is present, they again become motile. When an electric current is passed through a neutral suspension of spermatozoa in cane-sugar, the sperm travels rapidly to the positive pole where it accumulates[1]; round the negative pole, however, the sperma-

[1] This was also observed by R. S. Lillie (2) in the case of the spermatozoa of the frog.

tozoa become exceedingly active. The behaviour of the sperm round the negative pole is apparently due to the liberation of alkali, which can be detected by means of an indicator in the solution. If, however, spermatozoa are suspended in a faintly acid solution of cane-sugar, no migration takes place to the positive pole, and no activation was observed at the negative pole; in such a suspension, the electric current causes the sperm to form a retiform aggregation throughout the solution. These facts appear, then, to show that motile spermatozoa possess a negative charge on their surface, and that this charge is lost in the presence of free hydrogen ions.

(2) The Behaviour of Spermatozoa in the presence of Trivalent Ions.

The effect of simple trivalent kat-ions upon spermatozoa is very remarkable. If a drop or two of a very weak solution of cerous chloride is added to a suspension of Arbacia sperm in sea-water the spermatozoa become intensely active, and rapidly aggregate into clumps, which soon become visible to the naked eye. Examined under the microscope, these clumps are seen to consist of extremely active spermatozoa. After some time the aggregated spermatozoa become motionless, but those which are not aggregated continue to swim actively.

The "aggregation" by means of cerium appears to be partly reversible by mechanical agitation, if the aggregation has not existed for more than a few minutes. If a suspension of "aggregated" spermatozoa be agitated in a phial, the fluid becomes quite homogeneous to the naked eye. Under the microscope, however, small "aggregates" are still found to be present. After a few minutes, macroscopic aggregates again become visible. Finally, all the spermatozoa come to rest, and are mostly "aggregated" together into small clumps. It is remarkable that the final effects of the cerium ions[1] is the same as that of hydroxyl ions, viz. an

[1] The solution of cerium was not alkaline in solution, being faintly acid to neutral red and phenophthalein.

almost complete agglutination of all the spermatozoa. It resembles, however, the effect of the hydrogen ion in the formation of aggregated masses of living spermatozoa, although this phenomenon is very much less obvious in the case of the hydrogen ion.

If a piece of filter-paper previously soaked in a solution of cerium be put into a dilute suspension of sperm in sea-water, the spermatozoa are rapidly attracted to the paper. They aggregate round the edges and are at first exceedingly motile, after a time, however, they become quiescent, and the paper is covered by a thick felt of agglutinated sperm.

Experiments with neodymium nitrate gave results exactly similar to those with cerous chloride.

It is important to note that the effect of these trivalent positive ions is completely removed by means of sodium citrate. In other words, there is little or no doubt that these experiments afford another fact in favour of the belief that trivalent ions affect living organisms by virtue of their electrical charge.

Now, the "aggregation" phenomena of spermatozoa has been observed by F. R. Lillie (1) in the case of a suspension of *Nereis* sperm in normal sea-water. From his account I conclude that the behaviour of a normal suspension of *Nereis* sperm is very similar to that of *Arbacia* sperm in the presence of cerium ions. Lillie concludes that the behaviour in the case of *Nereis* sperm is due to positive chemiotaxis towards a CO_2 gradient. He also observed that sea-water which had been in contact with unfertilised eggs has a marked effect on the activity of spermatozoa. He found that such sea-water had a threefold action on the sperm: (1) It greatly increases their activity; (2) it aggregates them; (3) agglutinates them. Lillie regards these effects as due to an "agglutin" which has been extracted from the eggs with which the water was in contact. Further, this "agglutin" cannot be obtained from any other tissue except the ripe ovaries. From its properties it may be inferred that the substance is of a colloidal nature.

The effects of trivalent positive ions and of "egg-agglutins" upon living spermatozoa appeared to be so nearly identical that considerable care was taken to test this conclusion by the method of examination used by Lillie (1). The result of injecting a drop of cerous chloride or neodymium nitrate into a suspension of sperm under a cover-glass was apparently identical with that described by Lillie on p. 550 of his paper, when using egg-extractive. It should be noted also that both trivalent, positive ions and egg-extractive are efficient at extremely low dilutions.

It would thus appear that the behaviour of spermatozoa towards "agglutin" is identical with its behaviour towards a trivalent kat-ion.[1]

P.S.—It had been hoped that circumstances would permit of a more detailed investigation of the above facts in the near future. This, however, is no longer the case, and the present note is published merely to suggest that the nature of the electric charges upon the surface of the gametes may play an important rôle in the behaviour of these cells.

Note.—The inactivity of Luidia sperm in normal sea-water is all the more remarkable from the following considerations. The habitat of this species is given by Crawshay[2] as being 42–50 fathoms. Now, the hydroxyl-ion concentration of sea-water at this depth is appreciably lower than that of the surface water which was used in the laboratory. Unfortunately, a complete investigation of the alkalinity of the water in the English Channel is not available, but the available data afford no evidence for supposing that the alkalinity of the sea-water ever reaches the value which is necessary to effect activation of the sperm under experimental conditions. It would be exceedingly interesting to know whether the Luidia which are dredged from the Rame Eddystone grounds at Plymouth ever breed, or whether the limit of migration of this species is fixed by the alkalinity of the sea-

[1] I understand that Prof. Lillie intends to investigate whether the behaviour of spermatozoa to egg-extractives and to trivalent-positive ions are really identical phenomenon or not; further discussion of the point must be reserved until absolute certainty is established.

[2] 'Journ, M. B. A.,' vol. ix, p. 334, 1912.

water. It is almost certain that the true "home" of this Asteroid lies considerably further west of Plymouth.

Bibliography.

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